Synopsis of proposed thesis

Toxicological effect of Zinc and Lead on Biological and Biochemical Parameters of Fish *Oreochromis mossambicus* (Peters, 1852)

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1.Brief Resume of Proposed Work

Fish Oreochromis mossambicus is mostly used as food. It has nutritional value and it is commercial fish. It is easy to handle and maintain easily in laboratory condition. Zinc and lead are heavy metals which are toxic to fish and altimately affect the food chain. This research help to aware people about adverse effect of zinc and lead on fish. It will save The life of fishes, health of community and help to save biodiversity.

2.Introduction

Heavy metal causes pollution of soil, water. Heavy metal pollution causes serious problem to man, environment as well as all living organisms. Due to Industrialization, increase use of pesticides, fertilizers causes heavy metal accumulation in soil, water bodies. Heavy metal in more concentration causes adverse effect on aquatic ecosystem and bioaccumulation of heavy metal is very known in fish. In polluted water heavy metals are widely distributed. Some heavy metals are essential in trace amount for normal biological physiological activities of aquatic organism. Heavy metal show varied effect on body of Organism and also show toxic effect on body. Heavy metal in higher congratulation causes harmful effect on the blood and vital organ of fish.

Fish is major part of the human diet. Fish contains more amount of protein and low saturated fat and also content sufficient amount of omega 3 fatty acid. Which are known to be support good health. It is cultivated and consumed in large quantity of all over the world. Lipids are the most important constituent of fish. Lipid contain determine the fish meat quality. Fat content of fish are very variable from species to species and it is also change due to seasonal changes, physiology, feeding, habitats. As fish is most important and vital link in food chain of ecosystem. There should be protective management strategies, such as removing pollutants like heavy metals from natural system will be necessary to sustain our freshwater fisheries. Adversity of pesticides fertilizers, industry waste, their residues are present in wide variety of aquatic habitats.

Increase concentration of zinc lead affect the ecosystem, reproduction and behaviour by causing biochemical pathological and physiological change (Nagaraju B. et al. 2011). Increase in concentration of heavy metal zinc and lead have widespread effect on life history, physiology and behaviour of most freshwater organism including fishes Due to dissolve zinc and lead in water bodies it increases water pollution, decreases dissolve oxygen level and increases toxicity of pollutants. This could alter the quality of fish habitat, food webs, change in habitat availability is extencibly linked to quality of water. Genetic change also occurs due to polluted water.

Water is the only biological option for fish are unable to migrate. Increase dissolved lead, zinc in water body show impact on fisheries it make difficult for developing country to meet there food demand, due to this economic loss also occur. This polluted water due to Zinc and lead produce more powerful stress for fishes living in natural and artificial system. No distinct morphological abnormality occur but affect growth, chlorophyll, total DNA, RNA, protein. Affect activity of amino acid and coenzyme, protein, sulphur, phosphorus, nitrogen, oxygen Khulkar Prceti.

Some heavy metals are essential and do specific biological function they are copper zinc, iron, manganese, molybdenum, magnesium, calcium all are essential for animal except cobalt essential for plants. Lead, Cu, Cd toxic they inhibit enzyme by forming mercaptides with sulphadyl group which are responsible for catalytic activity. Zinc is essential for all known life of all living organisms. Zinc is act as co-factor for 10% of all proteins. Zinc is function as an para cellular signalling substance as well as intracellular signalling substance. For zinc protein may act as transporters, chelators, molecular are sensors.

Two large families of transporters Slc 30 ;Slc 39 which function as regulatory network for distribution of zinc throughout body and within cells change in concentration of liable zinc ions affect metallothionein and several proteins. They either activate or inhibit by change in concentration of liable zinc iron. By homeostatic processes uptake of Zn across gut and gill is regulated by these protein. Aqueous zinc binds with dissolved organic matter [DOM] ln water dominant inorganic zinc spaces is free zinc ion. In natural water total zinc concentration influenced by human activities. Free zinc ion in water is lethal to fish. Zinc toxicity is modified by DOM, calcium, pH in water and increase its lethal effect.

In natural water some permissible concentration of zinc is allowed by legislation. In acute zinc toxicity calcium uptake is inhibited by freshwater fish. Not more information about sub lethal toxicity of zinc to fish is available and mechanism also not known. In chronic exposure to zinc of freshwater fish show lethality sensitive endpoint. (Christer Hogstrand 2011). It is a well known that copper, nickel, zing essential element, required by a wide variety of enzymes and other cell components and having vital function in all living organisms, but very high intake can causes adverse health problem (Demirezen and Urue, 2006).

Different anthropogenic activities such as mining, smelting, use of zinc in dye casting, metals, plastic and alloys can direct its release toward aquatic ecosystem through various channels and stream. Zinc enter into the fish body through different pathways, including skin or general body surface, gill alimentary canal or gut by consumption of contaminated food. Toxic effect of excess zinc different in different types of species of fishes. Excess zinc destroys Fish gill tissue and ultimate effect that fish die. Acute concentration of zinc increases stress in fishes and it ultimately result in death of fish (Skidmore, J.F., 1964).

Lead is non- essential and toxic even in low concentration in human lead delays physical and mental health. In some species of fish lead observed maximum bioaccumulation in kidney. The water and sediment were the primary source of lead the reared fish in this pound also accumulate a high level of lead in different tissue. The maximum lead accumulation observed in kidney followed by liver, gills muscles in all the fishes. Abdel-Baki and co-workers also opened that kidney accumulate the highest concentration of lead while muscles accumulated the lowest (Abdel- Baki A. S. et al.2011). Lead does not induced metallothioein (MT) formation in tissues. It is also reported that lead accumulation does not very significantly with age and liver, kidney scales are the main site for a lead accumulation. Lead has industrial importance. Lead is main problem in aquatic system and lead comes from ore processing, smelting, refining operations, motor vehicle exhaust, agricultural runoff, domestic waste water effluents. Aquatic organism have ability to accumulate lead in tissue by exposure to polluted water containing high concentration of lead or by food. As more exposure to the high concentration of lead accumulate more lead in tissue of fish. When fish gills exposed to sublethal concentration of lead on surface of gill lamella. Gill epithelia are mainly affected by high concentration

of lead. Fish gills are & target organ of heavy metals i.e. lead. When lead is enter in the body then it enter into blood circulation & reached to the organs. Lead accumulate in kidney, lever, gills (Pretto et al.,2011) .

Lead causes oxidative stress and damage to they living tissue of animal and humans. More concentration of lead causes the intensive lesion to muscle tissues and also affect the affect to intestine and skeletal (Sharma et al. 2014). Many problems are cause in fishes due to exposure to heavy metals. Lead and other heavy metals causes the histopathological lesions in kidney, spleen and muscle (Authman et all 2012). Lead is general protoplasmic poison. It accumulate in a tissue and it act slowly on a body. Lead causes serious environmental hazards and also harmful effects on aquatic animals (Ashraf et al.,2012).

The homeostatic level of body is disturbed when lead is accumulated in a tissue. For example, aminole vulinate (ALA) is one of the intermediate products during heme synthesis. It is condensed by zinc containing enzyme ALA dehydrogenase which is very sensitive to inhibition by lead toxicity. This ALA accumulate in the cell and creates serious problems, that is neurological disorder, accumulation of lactate in a cell, low pH of the protoplasm and out flux of calcium from bone. Essential heavy metals are taken up by fishes and other aquatic organism from water, food sediments.

The natural balance of heavy metal concentration in water is highly disturbing with her increasing trend of industrial and aquacultural activities. The excessive uptake of essential and nonessential heavy metal causes its accumulation in the body organ and tissues. Consumption of such metal contaminated fish by human can cause serious health issue (Kamaruzzaman et al.2011)

Most of these heavy metals are characterised by being accumulation in tissue and lead to poisoning of fish. These metals can effectively influence the vital operation and reproduction of fish, weaken the immune system and induce pathological changes. As such, fish are used as bioindicators, playing an important role in monitoring heavy metal pollution. Finally some recommendations are given to treatment of different kinds of waste water, sewage and agricultural wastes before discharge into aquatic systems.

Also enforcement of laws and legislation regarding the protection of aquatic environment must be taken into consideration. Here *Oreochromis mossambicus* fish used

for study of toxic effect of lead and zinc on freshwater fish. *Oreochromis mossambicus* is a worldwide species it is form useful and available easily all over the world. It is important edible fish.

As *Oreochromis mossambicus* is the important communication commercial fish worldwide (El-sayed 2006). It is a bioindicator organism for study the toxicity and effect of lead and zinc and other heavy metals .*Oreochromis mossambicus* is easy to handle, easy to maintain in laboratory condition. It show prompt response to change the environmental conditions and adverse effect of polluted water. Zinc is important for a normal growth production and other process of fish (Eisler, 1993). Zinc is a toxic in high concentration (Bielmyer et al. 2012). Lead is also toxic for fish. During the acclimatization period, the fish were fed once daily with commercial pellet food compositions (with 20% crude protein, 4% crude fat, 5% crude fiber,12% crude ash and 10% crude moisture) . The water was change daily and fish showing any an usual performance where excluded. In acclimatization period, the water was daily check for ph, temperature, dissolved oxygen. Water was changed every 2 days and fish were fed 40 minutes before each water change. This research help to find the adverse effect of heavy metal pollution and it will helpful to aware of people to reduce the heavy metal pollution of lead and Zinc. At end of this research some suggestion are forward to governing body so that it makes some important changes in the legislation that can be forced to implement by the industries, other responsible bodies that introduce heavy metal polluted water into water bodies. This research ultimately helpful to save the aquatic life, human life and ultimately to save the ecosystem diversity.

Source of fish

Oreochromis mossambicus fish is collected from Loha Masay Bij Kendra, Gondgao, Nanded. For transportation five gallon bucket with tight lid or plastic bags from pet store use rubber bands to secure the bags for travel longer than hour use pour oxygen from local pet store is use or containers with lid or insulated container or picnic cooler or Styrofoam cooler are use.

3. Review of Literature

Ali Muhammad Yousafzai et al.2017.in his work finds following details. They examine and compared heavy metal bioaccumulation in Cyprinus carpio and Labia rohita netted from Sardaryab, a tributary of river Kabul. By using atomic absorption spectrometry we assessed different organs including livers, gills and muscles. Metals studied were chromium iron, zinc,lead and copper. Livers of both species showed higher concentration of metals while muscles showed the least amount.

Mohammed A. Aldoghachi et al.2015.in his work mention following details. Lead cause of oxidative stress and damage to living tissue of animals and humans. More concentration of lead causes intensive lesion to muscle tissue and also affect to intestine and skeletal (Sharma et al. 2014). Effect of heavy metal (lead) on tilapia fish. Many problem are cause in fishes due to exposure to heavy metals. Lead and other heavy metals causes the histopathological lesions in kidney, spleen and muscle. (Authman et al. 2012).

A.A.Abdel Warith et al.2011.In his paper gives following details. Additionally, we estimated whether zinc concentration and exposure period in fluence the degree and nature of histological changes in liver of exposed fish. Two hundred and forty fishes (the average weight was 24.31 ± 2.85 grams) were distributed randomly in twenty four glass aquaria (80L) with stocking density of 10 fish for each. Fish mortality was significantly increased in a dose dependent manner, without any significant effect of a exposure duration. Histological change in the liver include hepatocyte degradation nuclear pycnosis, cellular swelling and congesion of blood vessels. There was marked difference in these changes among the different treatment of zinc concentration in addition to the intensity of histological change were however influenced by extent of exposure period.

4.Objective of research

- To study, analyse, examine the toxic effect of zinc and lead on a freshwater fish *Oreochromis mossambicus.*

- To find out biochemical parameters carbohydrates, lipids, proteins like albumin, urea, serum protein, serum lipids, glycogen change due to effect of different concentration Zinc and lead, to find out the effect on physiological activities.

5.Methodology to be adopted

Due to more Industrialization, more use of fertilizers, pesticides contains zinc and lead and that goes in water bodies by directly or indirectly. To increase awareness of people, the present work will carried out on impact of zinc and lead on freshwater fish *Oreochromis mossambicus.*

- All the precaution laid down by APHA et al. 1998 are followed for maintaining the fish.
- Such acclimatized Fish will expose for 96 hours to zinc and lead separately 480 sc to lethal (LC50) sublethal concentration. If mortality occurred during experimental period, dead fish were remove immediately to avoid depletion of vital tissue like muscles brain, liver, gills and kidney.
- Total protein with estimated by the method of Lowry et al 1951 and other biochemical parameter will recorded according to standard methods used.

6. Importance of study

1)International status

Progress in the research of toxicity affect mechanism of heavy metals on freshwater organism and their water quality criteria in China. Ya-jun Hog et al.2020.In this research work following details are given. Water quality criteria the scientific basis for formulating water quality standards and environmental management practices. Due to the development of Urbanization and Industrialisation, the problem of heavy metal pollution has serious environmental problem. Heavy metal not only have major impact on aquatic organism but also seriously threaten human health.

However, the current environmental criteria refer to maximum value limitations of environmental factors in environmental media where harmful or detrimental effect are not produce on specific protection objects. This study reviewed the source, hazard level, toxic effect, mechanisms and current research status of china

water quality criteria for heavy metal pollutants. In addition, the focus and direction of future research on the toxic effect of heavy metal on aquatic organism and necessary criteria changes were discussed. The present study present study would provide an important theoretical basis for future research of water quality criteria and risk assessment of heavy metal pollutants.

Ultrastructure effect on gill tissue induce in red tilapia Oreochromis species by waterborne lead exposure. Mohammed A Allographs et al.2016.Saudi Journal of Biological Science.In this research work following details are given. Experiments on hybrid red tilapia Orechromis species were conducted to assess histopathological effect induced in gill tissues of 96 hour exposure to water borne lead (5.5 mg/L). These tissues were investigated by light and scanning electron microscopy.

Result shown that structural design of gill tissues was noticeably disrupted. Major symptoms were change in epithelial cells, fusion in adjacent secondary lamellae, hypertrophy and hyperplasia of chloride cells and coagulate necrosis in pavement cells with disappeared of its macroridges. Electron microscopic X ray macroanalysis of fish gill exposed to sublethal lead revealed revealed that lead accumulated on surface of the gill lamella. This study confirmed that lead exposure incited a different of histological impairment in fish, supporting environmental water over aquatic system when polluted by lead.

Accumulation of some heavy metals and biochemical alteration in muscles of *Oreachromis niloticus* the from the river Nile in upper Egypt. Amal M. Yacoub et al. 2009.Present study aim to evaluate level of some heavy metals (Cu, Mn, Pb and Zn) in certain tissues (gills, intestine, muscles) of *Oreochromis niloticus* collected from different site of River Nile at Upper Egypt during winter and summer (2009).

Moreover the effect of accumulated heavy metal on total protein, total lipids and activities of transaminase enzyme (ALT and AST) in the fish muscles were studied. The obtained results revealed that the abundance of heavy metals in fish organ follow the order Mn>Zn>Pb>Cu. The concentration of cupper in fish muscle were below the maximum permissible limit. Total proteins, total lipids and the activities of ALT and AST where significantly lower in the muscle of studied fish.

2) National status

Following is work done on National level/ status

Elimination of essential (Cu, Zn) and nonessential (Cd, Pb) metals from tissue of freshwater fish Tilapia zilli. Mustafakalay et al. 2000.In this paper gives following details. Tilapia zilli were exposed to the small concentration (1 mg/L) of essential (Cu, Zn) and nonessential (Cd, Pb) metals for 10 days so that these metals would accumulated in the liver, gill, brain and muscle tissues.

Subsequently, the animals were transferred to uncontaminated water for period of elimination, during which sample were taken at day 1,7,15 and 30 Cadmium and lead accumulator in all the tissue and the tissue concentration increase many times compared to the level of these metals in the control fish (<0.05 lg /gd. W.). The brain also accumulated lead to a high level.

Copper also accumulated significantly ($P<0.01$) in all the tissue studied except for muscle tissue. The accumulation of zinc was not significant ($P >0.05$) in any tissues. The accumulation of the metal were found to be considerably different when the tissues and the metal were compare with each other. Lead was the only metal that was eliminated to a significant extent from the liver. Elimination of metal was showed considerable different in term of both the tissues and metals.

Growth, biochemical variables and Zinc bioaccumulation in Nile tilapia. Oeochromis niloticus (L) as affected by water borne zinc toxicity and exposed period – Mohsen Abdel T. et al. 2016.The present study was carried out to investigate the effect of sublethal zinc concentration on the growth performance, biochemical variables and Zinc in various organs of Nile tilapia Oreochromis niloticus (L) fish (25 ± 0.59 g) were exposed to 0.0,3.5, or 7.0 mg Zn per litre for the 1 or 6 weeks.

Fish growth was significantly reduced with increasing zinc concentration. Furthermore, glucose aspartate aminotransferase (AST), alanine aminotransferase (ALT) creatinine, and cortisol increase significantly with increasing zinc concentration and exposure time, with maximal values in the 7.0 mg zinc per litre treatment after 6 weeks. There was no significant change in whole body moisture content of zinc due to zinc exposure, although crude protein and total lipid content decrease significantly with increasing zinc concentrations.

Zinc Residue after one week were found to be follow the order of Gill >liver> kidney >muscles, meanwhile after 6 weeks it followed the order of liver >kidney> Gill >muscles. The present findings revealed the liver and kidney tissues are the prime side of a zinc bioaccumulation, while zinc load in the muscle was far low as compared to other organs.

3)Reginal Status

Godavari river is polluted due to many pollutents it also include heavy metals like zinc and lead. These heavy metals affect badly to aquatic animals like fishes. Oreochromis mossambicus is fish used for commercial purpose and it is also affected due to heavy metals like zinc and lead.

This research makes awareness in society to lower water pollution due to heavy metals. It will help to save the life of aquatic animals.

4) Scope of Study

It help in people awareness lowering use or no use of heavy metals or use substitute for these heavy metals. This research will helpful to encourage to use of atoms or product do which do not harm to any living organisms or ecosystems do not harm to aquatic organisms also.

Worldwide use of heavy metal zinc and lead may affect all water bodies and make them polluted and ultimately affect the community health of aquatic organisms also human community.

Increase in water pollution due to increase use of zinc and lead and other compounds. Since the early century zinc and lead pollution increases 4% as that of previous year about two third increase occurring since 1980.

Many international groups of controlling pollution due to zinc and maintain ecosystem free from their hazards. As water pollution due to zinc and lead is very common phenomenon where other factors also contribute in water pollution.

Water is life of living organism. It is a universal solvent at all animal bodies. Water has many important physical and chemical characteristics. The pollution

free water play major role as environmental parameters that affect and alter the Physiology, life activities and behaviour of aquatic organism. Such more concentration of lead and zinc affect not only large water bodies like sea and ocean but also smaller water bodies, including rivers and pond

Pollution due to more amount of zinc and lead several threats the distribution of aquatic animal, the animal biodiversity and impact of environmental change on water resource hence the attention should be given this topic for betterment of human life.

Currently our primary research objective is to encourage and assist the development of better and faster measures of environmental activity. We believe we can contribute directly as opposed to through highlighting the work of others, we are producing our own measure of lowering water pollution ultimately save ecosystem of earth.

This research making awareness in society to decrease use or no use of heavy metal that badly affect the water bodies. Instead of these toxic products use other natural protective products. Ultimately we save ecosystem of the earth to help sustain our freshwater fishery. This research work will be helpful to save the life of aquatic organism.

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7) Proposed work plan -with a tentative chapter scheme

1)Introduction

2)Preface

3)Toxicity effect of Zinc and Lead

4)Biological and Biochemical effect of Zinc and Lead

5)Literature Review

6)Geological Study Area

7)Material and Methods

8)Results and Discussion

9)Summary and Conclusion

10)References

8) Year wise plan off work and targets to be achieved

- **In first year**

The present work will be based on impact of more concentration of zinc and lead in the water that increases water pollution on same physiological activities which will be study in a first year of research. physiological activities like

Growth

Respiration,

Reproduction study across different

concentration gradients.

- **Second year**

Toxicity and biochemical haematological parameters are study in second year. Toxicity measurement and effect of Heavy metals Zinc and Lead on biochemical parameters are to be studied

- **In third year**

Statistical studies, calculations, results are to be depicted and thesis writing and submission of final synopsis and thesis will be done in third year.

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